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Molecular Marker Diversity and Field Performance in Commercial Cotton Cultivars Evaluated in the Southwestern USA

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ABSTRACT

Genetic diversity in modern upland cotton cultivars (*Gossypium hirsutum* L.) is thought to be narrow, thus limiting genetic advance. Robust information on the genetic relatedness among currently grown cotton cultivars is lacking. The objectives of the present study were to field test a sample of elite commercial cotton cultivars, including many transgenic cultivars representing the major cottonseed companies, and to evaluate their genetic divergence using simple sequence repeat (SSR) markers. Eighty-eight SSR primer pairs were chosen for genotyping that provided 177 SSRs. Jaccard's genetic similarity coefficients among 24 genotypes ranged from 0.694 to 0.936, with an average of 0.772, indicating that sufficient genetic diversity does exist within our sample of commercial upland cotton. Genetic similarities among cultivars from the same seed companies were generally higher than the mean of all cultivars and grouped into six major groups: two Deltapine (DP), one Stoneville (ST), one FiberMax (FM), and two New Mexico (NM) Acalas. One California Acala cultivar of New Mexico origin, developed by Phytogen (PHY), did not group with New Mexico Acala germplasm. Texas High Plains stripper type cultivars were distant from picker types and formed independent groups. Under New Mexico growing conditions, DP and ST cultivars yielded higher but produced lower fiber quality, while NM Acala cotton had lower yield but higher fiber quality. The PHY and FM cultivars were intermediate in cotton yield and fiber quality. Six SSR markers were identified to be significantly correlated with fiber yield or quality among the cultivars tested, providing impetus to validate the marker–trait associations.

Historically, germplasm in cotton breeding had been openly shared between public and private breeders in the USA, but this exchange of germplasm has been curtailed in recent years as public breeding programs were eliminated and seed companies assumed the nearly exclusive role of cultivar development. Presently, the focus of the few remaining public breeding programs has shifted to germplasm development and developmental breeding, while commercial cultivars have been solely released through seed companies. A number of studies have suggested that cultivated upland cotton germplasm possesses a low level of genetic diversity when evaluated by isozymes, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restricted fragment length polymorphism, and SSRs (Wendel et al., 1992; Tatineni et al., 1996; Pilley and Myers, 1999; Abdalla et al., 2001; Iqbal et al., 2001; Gutierrez et al., 2002; Lu and Myers, 2002). Genetic distances could be as low as 1 to 3%. Research in Australia, China, and Pakistan obtained similar results (Multani and Lyon, 1995; Zuo et al., 2000; Rahman et al., 2002).

The hypothesized narrow genetic base of upland cotton germplasm used in breeding has been considered as one of the reasons contributing to the lack of progress in the improvement of cotton cultivars to meet the needs of cotton growers and industry in the USA during the last 15 yr (Meredith, 2000; Lewis, 2001). A series of studies on pedigrees, coefficients of parentage (CPs), and genetic diversity for 260 cotton cultivars released in the USA between 1970 and 1990 were conducted (Bowman et al., 1996, 1997; May et al., 1995; van Esbroeck et al., 1998; Bowman et al., 2003). The mean CP suggested wide genetic diversity among the 260 cultivars (Bowman et al., 1996), but that diversity was declining due to the frequent use of a few parents combined with reselection within cultivars and elite germplasm to develop new cultivars (van Esbroeck et al., 1998). Surprisingly, the introduction of transgenic cotton cultivars has significantly reduced field genetic uniformity since the percentage of the crop planted to a few cultivars has declined (Bowman et al., 2003), even though all the transgenic cotton cultivars were developed via backcrossing using popular nontransgenic cultivars as recurrent parents. However, multivariate analysis of agronomic and fiber traits of ancestral cultivars detected high similarity (van Esbroeck et al., 1999), supporting the conclusion that modern cotton cultivars have a narrow genetic base when evaluated with isozyme and DNA markers (Wendel et al., 1992). This suggested that pedigree analysis may overestimate genetic distance among modern cultivars (van Esbroeck et al., 1999). Therefore, genetic diversity among modern commercial cotton cultivars needs to be assessed using more precise methods.

It is known that cotton cultivars released by different developers and adapted to the same region appear highly morphologically similar, but they could perform very differently from unadapted cultivars introduced from other regions or countries. For example, New Mexico Acala cultivars, known for their high fiber quality, good Verticillium wilt (*Verticillium dahliae* Kleb) tolerance, and large boll size (Smith and Cothern, 1999), are adapted to the southwestern growing region of the U.S. Cotton Belt. Even though they are very tall and late maturing with low yield when grown in other regions

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Abbreviations: AFLP, amplified fragment length polymorphism; CP, coefficient of parentage; DP, Deltapine; FM, FiberMax; JC, Jaccard's coefficient; NM Acala, Acala of New Mexico origin; PHY, Phytogen; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; SM, simple match coefficient; SSR, simple sequence repeat; ST, Stoneville.

such as the Midsouth, they have been used extensively as parental lines for developing other types of cotton cultivars since the 1950s (Bowman et al., 1996). However, genetic information on the relatedness of the commercial cotton cultivars from different sources and the Acala cottons is still lacking.

The uniqueness of the Acala cotton is perhaps mostly due to its breeding history, in which germplasm from *G. barbadense* L. and Triple Hybrid (*G. arboreum* L. × *G. thurberi* Todaro × *G. hirsutum*) was introgressed (Smith and Cothern, 1999). Interspecific introgression was also evident in the development of high quality Pee Dee germplasm lines (May, 2001). There have been attempts in introducing fiber quality genes from Acala and/or Pee Dee lines into other cottons to develop high-yielding cultivars, but success in improving fiber quality has been limited (Bowman and Gutierrez, 2003). The priority in Acala cotton breeding programs has been fiber quality, including fiber length, strength, and fineness, that has maintained and accumulated desirable genes for fiber quality. On the contrary, focus on high yield and wide adaptation in other breeding programs has not simultaneously improved fiber quality. This practically divergent selection has lead to cultivar development in separate directions, which could have established linkage disequilibrium between fiber quality or yield genes and DNA markers. Therefore, the genetic diversity assessment between commercial Acala and other cottons based on molecular markers could provide clues in identifying chromosomal regions in Acala cottons that might be associated with their agronomic performance.

The objectives of our study were to (i) estimate genetic diversity among newly released commercial cotton cultivars planted to 37% acreage in the USA using SSR markers; (ii) evaluate genetic relationships between commercial cotton cultivars of various origins and New Mexico Acala cultivars; and (iii) identify SSR markers potentially associated with lint yield and fiber quality.

MATERIALS AND METHODS

Plant Materials

Twenty three commercial cotton cultivars, including Acala 1517-95, Acala 1517-99, Acala 1517-02, and Acala 1517-03, that were tested in the National (or Regional) Variety Trial in New Mexico in 2001–2002, were selected for this study (Table 1). Many cultivars contained Bt (Bt, lepidopterous larvae resistant; Bollgard, Monsanto Corporation, St. Louis, MO) and glyphosate [*N*-(phosphonomethyl)-glycine] resistant Roundup Ready (Monsanto Co., St. Louis, MO) genes. Collectively, these cultivars were planted to 37% of cotton acreage in the USA in 2004 (National Cotton Council of America, 2005). TM-1, the genetic standard of upland cotton (Kohel et al., 1970), was also included for comparison purposes.

Simple Sequence Repeat Fingerprinting

Leaf tissues from at least 10 plants per line were harvested from the field plots in November 2002. Genomic DNAs were extracted from the bulked leaves of each of the 24 genotypes using the protocol by Zhang et al. (2000) or the Qiagen DNeasy Plant Mini Kit (Qiagen, Santa Clara, CA) following the manufacturer's instructions. The DNA concentration was determined by TD-360 Mini-Fluorometer (Turner Designs, Sunnyvale, CA).

Eight-eight pairs of BNL SSR primers, labeled with fluorescent HEX (4,7,2′4′5′7′-hexacloro-6-carboxyfluorescein), NED (7′8′-benzo-5′fluoro-2′4,7-trichloro-5-carboxyfluorescein), or FAM (6-carboxyfluorescein), were selected for the present study. On the basis of Liu et al. (2000b), these SSR primers were chosen to amplify fragments that were distributed on most of known chromosomes with two to four markers per

Table 1. Cotton genotypes tested, their hectarages planted in the USA in 2004, and pedigree information developed from various seed companies.

Genotype	U.S. hectarage†	Developer	Pedigree
	%		
TM-1	0	Kohel et al. (1970)	Inbred line from DP 14
DP 458 BR	1.52	Delta and Pine Land Co.	DP 5415‡ (DP 50/DP 90)
DP 449 BR	2.04	Delta and Pine Land Co.	DP 5415/DP 5690
DP 491	0.07	Delta and Pine Land Co.	DP 5415/DP 2156
DP 555 BR	14.89	Delta and Pine Land Co.	Delta Pearl/DP 655 BR (DP 5690)
SG 125 BR	0.10	Delta and Pine Land Co.	DP50/DES 119
PHY 78	0.26	Phytogen	NM B2541/B3112
PHY 72	1.45	Phytogen	Acala Prema/Acala 1517D
FM 991 R	0.37	Bayer Crop Science	unknown
FM 989 BR	1.87	Bayer Crop Science	FM 989 recurrent parent
FM 989	1.91	Bayer Crop Science	unknown
ST 5599 BR	5.11	Stoneville Pedigreed Seed Co.	ST LA887 recurrent parent
STX 0003	0	Stoneville Pedigreed Seed Co.	unknown
ST 4793 R	1.29	Stoneville Pedigreed Seed Co.	ST 474 recurrent parent
ST 580	0	Stoneville Pedigreed Seed Co.	ST 468/DP 5415
ST 457	0	Stoneville Pedigreed Seed Co.	ST 468/ST LA 887
BXN 49B	0.09	Stoneville Pedigreed Seed Co.	ST 474 recurrent parent
ST 4892 BR	4.21	Stoneville Pedigreed Seed Co.	ST 474 recurrent parent
1517-95	0	New Mexico State University	From 1517-E2 (3080/PD2165)
1517-99	0.05	New Mexico State University	B742/E1141
1517-02	0	New Mexico State University	Prema//Acala 1517-95/GC-362
1517-03	0	New Mexico State University	B4222/H1014
NX 2419	0	Syngenta	unknown
All Tex Atlas	0.75	All Tex	CA3006/Paymaster HS26

† Based on National Cotton Council of America (2004).

‡ Indicates that this cultivar was the recurrent parent used in development of this transgenic cultivar. Pedigree of the recurrent parent or recurrent parent is given in parentheses.

chromosome. This ensured broad genome coverage of genotyping for representational estimation of genetic distance. The PCR reactions were performed with a thermal cycler (PerkinElmer 9600 Thermocycler) in a 10-μL reaction solution containing 80 ng of DNA template, 0.15-μ*M* primers, 0.2 m*M* each dNTPs, 1 × GeneAmp PCR Buffer, 2.5 m*M* $MgCl_2$, and 0.5 units of AmpliTaq DNA polymerase (PerkinElmer, Foster City, CA). The PCR conditions were as follows: 7 min at 95°C, followed by 40 cycles of 15 s at 94°C for DNA denaturing, 30 s at 55°C for primer annealing, and 2 min at 72°C for extension with a final extension for 30 min at 72°C. The finished PCR samples were stored at −20°C until use.

The PCR products were separated by polyacrylamide gel electrophoresis using an ABI377 Sequencer (PerkinElmer; Liu et al., 2000a). Most SSR primers usually amplified one or two major bands, while some gave more than two bands. For the SSR markers, all the alleles were treated independently as a binary variable with 1 for presence and 0 for absence, because heterozygous status for codominant markers in the true breeding cultivars or lines was very rare, if any. Genetic similarity coefficients were calculated based on simple match coefficients (SM) and Jaccard's coefficient (JC) using the Numerical Taxonomy Multivariate Analysis System (NTSYSpc) Version 2.1 software package (Exeter Software, Setauket, NY). The resulting similarity coefficients were used to perform the cluster analysis using the unweighted pair group method of arithmetic means (UPGMA).

Field Trials

In 2001 and 2002, the cotton cultivars were tested at the Layendecker Plant Science Center, near Las Cruces, NM, a typical Acala cotton growing environment. The cultivars were arranged in a randomized complete block design with four replications. The plot size was two rows spaced 1.3 by 15.2 m. When cotton was mature, 50 sound, open bolls were hand harvested from each plot for measuring lint percentage and fiber quality. Fiber quality traits including length, strength, and micronaire were tested using in-house single instruments (Fibrograph, Micronaire, and Stelometer). Then, each of the plots was mechanically harvested for seed-cotton yield. The data were subjected to ANOVA using AgroBase 21 (Agronomix Software Inc., MB, Canada). The means of fiber yield and quality from cultivars common in 2 yr were used to conduct correlation analysis with the SSR markers using AgroBase 21. The correlation is equivalent to single factor regression analysis or *t* test when genotypes are grouped into two groups, that is, presence vs. absence of individual SSR markers.

RESULTS AND DISCUSSION

Genetic Diversity among Commercial Cotton Cultivars

The eighty-eight pairs of BNL SSR primers produced 177 alleles among TM-1 and 23 commercial cotton cultivars. These SSRs are distributed on 45 linkage groups with two to four loci per chromosome, with 43.0 and 36.1% of the SSRs on subgenomes A and D, respectively, whereas the remaining SSRs could not be assigned to chromosomes or subgenomes (Liu et al., 2000b; Lacape et al., 2003). The major SSR bands amplified were in good agreement with previously published results (Liu et al., 2000b). Genetic similarities based on the JC among the 24 genotypes ranged from 0.694 to 0.936, with an average of 0.772. This was toward the low end of the published results on genetic similarities among upland cotton. Other genetic diversity estimates in cotton have been reported using RAPD markers to range from 1 to 8% among Australian cultivars (Multani and Lyon, 1995) and 2 to 7% among 10 influential U.S. cotton germplasm lines (Lu and Myers, 2002). However, Gutierrez et al. (2002) reported genetic distances as high as 10 to 22% among five U.S. upland cotton cultivars, four Australian-bred cotton cultivars, and two converted, day-neutral lines evaluated by SSR markers. In our test, we chose SSR markers (about 40% of the BNL SSR markers) that produced higher polymorphism within upland cotton based on our studies. Thus, the genetic similarity obtained is not as high as these tests that used randomly chosen markers, which may more accurately estimate genetic similarities if they are evenly distributed across the cotton genome. The selection of markers could produce bias in overestimating the genetic diversity, but the tendency of genetic relationships between the germplasm tested in the present study should not be changed. Furthermore, methods in estimating genetic distances also make a difference. The SM considers both presence and absence of a fragment as shared markers among genotypes, resulting in higher estimates on genetic similarities; whereas JC only includes the presence of a fragment in its calculation with absence of the band excluded, since absence of a band is, understandably, the result of lack of priming site(s) and does not mean that they are similar among some genotypes. Despite the fact that JC is preferred, SM is still widely used for various markers, including SSR and RAPD in many crops (Li and Nelson, 2001; Warburton et al., 2002; Fu et al., 2003). In our study, we included both measures for comparison purposes and for inference of genetic relationships among cotton genotypes. Because of the codominant nature of many SSRs, absence of an allele is an indication of similarity among genotypes. Therefore, SM should also be an appropriate index of genetic distance.

Similarity among cultivars from the same seed company was generally higher than that among cultivars from different sources (Table 2). For example, cultivars from Delta and Pine Land Company (DP), Stoneville Pedigreed Seed Company (ST), and Bayer Cotton Seed International (FM) had average similarity coefficients of 0.854, 0.863, and 0.886, respectively. On average, cultivars from ST were most similar to those from DP (0.832) and FM (0.833). Acala cultivars were the most distant from the others (Table 2). Among the six cultivars tested from DP, DP 458BR, DP 555BR, and SG 125BR were similar, with similarity coefficients ranging from 0.881 to 0.906. DP 458BR and SG 125BR shared cv. DP 50 in their pedigrees. However, their high similarity with DP 555BR was unexpected because the latter was developed in Australia. DP 555BR and DP 449BR shared 'DP 5690' in their pedigree (Table 1), but they were grouped separately. The results illustrate that pedigree information or geographic origins of cultivars may not accurately reflect genetic relatedness among genotypes, whereas DNA markers could better reveal the genotypic relationships when there are sufficient markers and they are distributed across all chromosomes.

Table 2. Range and average genetic similarity based on Jaccard's coefficient between cultivars of different sources.

Comparison between cultivar sources	Similarity coefficient	
	Range	Average
Within Delta and Pine Land Seeds (DP)	0.800–0.906	0.854
Within Phytogen (PHY)	0.819	0.819
Within FiberMax (FM)	0.871–0.915	0.886
Within Stoneville Pedigreed Seeds (ST)	0.808–0.936	0.863
Within New Mexico Acala (NM)	0.706–0.891	0.796
Between DP and PHY	0.774–0.839	0.802
Between DP and FM	0.768–0.890	0.803
Between DP and ST	0.771–0.892	0.832
Between DP and NM	0.702–0.845	0.772
Between DP and NX	0.765–0.829	0.801
Between DP and All Tex Atlas	0.721–0.756	0.743
Between PHY and FM	0.694–0.840	0.765
Between PHY and ST	0.756–0.859	0.806
Between PHY and NM	0.728–0.890	0.781
Between PHY and NX	0.762–0.805	0.784
Between PHY and All Tex Atlas	0.744–0.785	0.765
Between FM and ST	0.792–0.886	0.833
Between FM and NM	0.735–0.855	0.802
Between FM and NM	0.756–0.824	0.796
Between FM and NM	0.716–0.740	0.730
Between ST and NM	0.724–0.838	0.779
Between ST and NX	0.765–0.823	0.796
Between ST and All Tex Atlas	0.718–0.756	0.733
Between NM and NX	0.802–0.843	0.820
Between NM and All Tex Atlas	0.733–0.772	0.750
Between NX and All Tex Atlas	0.769	0.769

Furthermore, these three cultivars were equally related to TM-1 with pairwise similarity coefficients of 0.852 to 0.894 with TM-1. TM-1 was developed by many generations of self-fertilization from DP 14 (Kohel et al., 1970). The other two DP cultivars, DP 449BR and DP 491, which shared 'DP 5415' in their pedigrees (Table 1), were similar to each other with a similarity coefficient of 0.895, but were least similar to the other DP cultivars. The same conclusions regarding genetic diversity were found with SM, but the estimates were higher than JC (data not shown).

The two Phytogen Seed Company (PHY) cultivars had moderate genetic similarity (JC = 0.819). The three FM cultivars were highly similar (mean JC = 0.886). Surprisingly, FM 989BR, the transgenic version of FM 989 containing Bt and R genes from Monsanto, was not as similar (0.916) to its recurrent parent, FM 989, as expected. Among seven ST cultivars, ST 5599BR was highly similar (0.900) to ST 457 since they had one parent ('ST LA887') in common; ST 457 was also highly similar (0.885) to ST 580 since they shared 'ST 469'; 'BXN 49B' and 'ST 4892BR' had the highest similarity (0.936), and both were also highly similar to 'ST 4793R' since 'ST 474' was used as the recurrent parent in developing these three transgenic cultivars (Table 1). Surprisingly, ST 4793R was not as close to ST 4892BR (0.897), as expected, since the two were developed from ST 474 as the recurrent parent. Perhaps the different parents that donated the Bt and glyphosate resistance genes contributed to the genetic diversity between ST 4793R and ST 4892BR. STX 0003 was the most distant from the other ST cultivars, with similarity coefficients ranging from 0.795 to 0.840.

Interestingly, the New Mexico Acala cultivars were unexpectedly dissimilar to one another, with JC ranging from 0.706 to 0.891. However, Acala 1517-99 was highly similar to Acala 1517-95 (0.891) since both had Acala 9130 as a common ancestor. Both were also similar to 1517-03 (0.845 and 0.857, respectively). But, these three were dissimilar to 1517-02 (0.706 to 0.744), which is highly similar to 'PHY 78' (0.890). Another California Acala cotton cultivar, PHY 72, was neither highly similar to PHY 78 (0.800), nor to New Mexico Acala cotton (0.759 to 0.808), even though it was developed using New Mexico Acala 1517D as one parent. Two other cultivars, NX 2419 and All Tex Atlas, developed on the Texas High Plains as stripper-type upland cotton, were not similar to any other cultivars (Table 2).

On the basis of the JC coefficients, the 24 genotypes can be classified into six major groups (Fig. 1): (i) DP group I: includes TM-1, DP 458BR, DP 555BR, and SG 125BR; (ii) DP group II: includes DP 491 and DP 449BR; (iii) ST group: includes ST 5599BR, ST 457, BXN 49B, ST 4892BR, ST 580, and ST 4793R; (iv) FM group: includes FM 989, FM 989BR, FM 991R, and STX 0003; (v) Acala group I: includes Acala 1517-95, 1517-99, and Acala 1517-03; (vi) Acala group II: includes PHY 78 and Acala 1517-02; (vii) Acala PHY 72; (viii) NX 2419; and (ix) All Tex Atlas.

Most DP and ST cultivars each formed a separate group, as expected, due mainly to the repeated use of in-house germplasm. These two groups are genetically closer since they formed a large class before grouping with other DP, ST, and FM cultivars (Fig. 1). Unexpectedly, even though many cultivars from the same seed company were genetically similar, some cultivars from different companies were grouped together, perhaps due to the utilization of similar parental sources. For example, STX 0003 was grouped together with the three FM cultivars, which in turn were grouped with DP 449BR and DP 491. However, DP 449BR and ST 580 had a common parent (DP 5415), and they were not grouped together. Interestingly, the two California Acala cotton cultivars PHY 72 and PHY 78 and four NM Acala cultivars were not grouped together; instead they formed three separate groups. Acala 1517-02 had 1517-95 in its pedigree, but the two did not group together (Fig. 1). In general, NX 2419, Atlas, and Acala cottons were the most genetically diversified from other cultivar sources and could be important sources for new cultivar development if they differ in useful agronomic traits.

It should be noted that cultivar grouping here by cluster analysis was based on the polymorphic SSRs that did not have a full coverage of cotton genome. Cultivars grouped together by the SSRs could have noticeable phenotypic differences in morphology, growth habits, and agronomic traits. Therefore, a genome-wide survey could provide a more representative picture of genetic and phenotypic diversity among cotton genotypes. However, considering that the cotton genome is estimated to contain 2200 Mb in size and is about 5400 cM in recombinational length, it would require more than 500 markers evenly distributed on the genome for coverage of 10 cM per marker. At present, this number is still an unrealistic task for cotton.

In summary, 88 pairs of SSR primers were used to

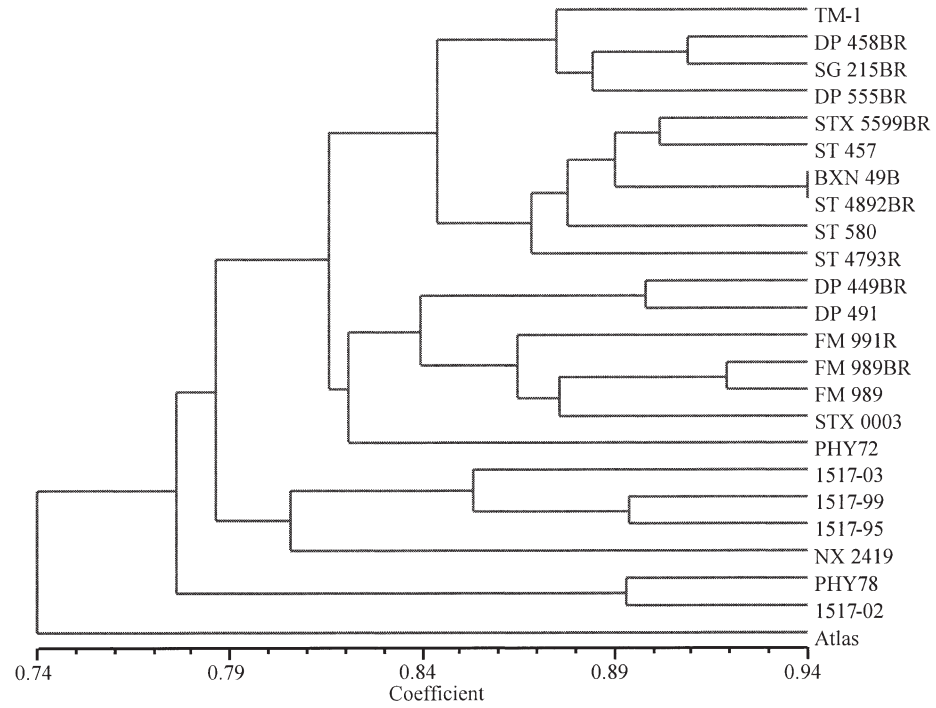

Fig. 1. A dendrogram of 23 commercial cotton cultivars and TM-1 by the unweighted pair-group method, arithmetic average (UPGMA) procedure based on Jaccard's coefficient from 177 SSR marker alleles.

amplify 177 SSRs in TM-1, and 23 commercial upland cottons were developed from four major cottonseed companies and the New Mexico cotton-breeding program. Significant genetic diversity appears to exist among the commercial cultivars based on the SSR marker polymorphism. Many cultivars from the same seed company were similar, but high similarity could be identified from cultivars from different seed sources. Two Texas High Plain Stripper type cultivars (NX 2419 and All Tex Atlas) were distant from the Picker type cultivars. The New Mexico Acala cultivars, together with the two Stripper type cultivars, were the most distant and diverse and should receive attention in the future research and breeding effort as sources of genetic diversity. The marker diversity between several pairs of isogenic cultivars (e.g., FM 989 vs. FM989BR, and ST 474 vs. ST 4793R) with and without Bt or/and R genes indicates that the transgenic cultivars were not as related as expected to their respective conventional cultivars.

Field Performance

Generally, midsouthern cultivars developed by ST and DP were higher in yield but had lesser fiber quality compared with Acala cotton types. On the other hand, New Mexico Acala cotton yielded less, but produced high quality fiber. On the basis of the 2001 and 2002 field tests (Table 3), New Mexico Acala had fiber length of 30.7 to 32.0 mm, with fiber strength of 227.4 to 255.8 kN m kg^{-1} and average micronaire readings of 4.4. Most DP and ST cultivars produced fiber shorter than 30.5 mm, with fiber strength ranging from 175.4 to 215.6 kN m kg^{-1} and micronaire readings of 4.0 to 4.6. The FM and PHY cultivars had an intermediate fiber quality, with fiber length of 29.2 to 30.7 mm, strength of 212.7 to 242.1 kN m kg^{-1}, and micronaire readings of 4.3 to 4.7.

Fiber length, strength, and micronaire reading were closely or significantly correlated in that cultivars with longer fiber usually had stronger fiber and lower micronaire reading. Also, cultivars having stronger fiber tended to have lower micronaire reading. These fiber quality traits were not significantly correlated with fiber yield (Table 4).

Correlation between SSR Markers and Fiber Yield and Quality

The informative SSR markers were used in correlation analysis with lint yield and fiber quality (Table 5). Among six SSRs that were correlated with fiber yield or quality ($P < 0.05$), three SSRs, BNL1694-252, BNL2634-254, and BNL3649-193, were significantly correlated with lint yield. The marker frequencies between Acala and non-Acala groups were significantly different. For the SSRs BNL1694-252 and BNL2634-254, 1/3 of the Acala cultivars carried these SSRs, while only 1/6 in the non-Acala group had the markers. For BNL3649-193, only one cultivar in the Acala group (1/6) carried it, while it was present in 13 of the 18 non-Acala cultivars. The presence of BNL1694-252 and BNL2634-254 was significantly correlated with reduced lint yield, while presence of BNL3649-193 was correlated with higher yield. BNL1694-252 and BNL2634-254 are located on Chromosome C7 and D02, respectively (Lacape et al., 2003; Nguyen et al., 2004).

For fiber quality, the presence of BNL3590-190 and BNL3792-235, located on Chromosomes C2 and A02, respectively (Lacape et al., 2003), was correlated with

Table 3. Lint yield and fiber quality in selected upland cotton cultivars grown near Las Cruces, NM, in 2001 and 2002.

Cultivar	Lint yield		2.5% span length		Fiber strength		Micronaire	
	2001	2002	2001	2002	2001	2002	2001	2002
	kg ha^{-1}		mm		kN m kg^{-1}			
1517-99 (check)	1997.28	1611.46	32.0	31.0	249.2	238.9	4.03	3.98
1517-03	2311.19*	1590.59	31.5	31.0	227.4*	236.7	4.58*	3.88
1517-02	2088.24	1710.55	32.0	31.0	255.3	252.9	4.60*	4.08
PHY 72	2331.42*	1962.70*	30.7*	30.5	233.1*	239.6	4.73*	4.40*
PHY 78	1814.78	1796.24	29.2*	29.0*	222.2*	235.1	4.33*	3.98
FM 989	2561.15*	1269.67*	29.7*	30.0*	233.3*	225.8	4.45*	4.15
DP 555 BR	2651.88*	2078.38*	30.0*	29.2*	192.3*	212.4*	4.48*	4.15
DP 491	2552.22*	1806.88	32.0	30.7	219.4*	220.8*	4.40*	4.00
SG 125 BR	2273.33*	2102.44*	27.9*	26.9*	175.48	169.9*	5.10*	4.65*
All-Tex Atlas	1340.63*	1111.92*	27.4*	27.4*	207.3*	213.4*	4.83*	4.58*
ST 4892 BR	2310.17*	2095.33*	29.5*	27.9*	197.1*	196.6*	5.28*	4.83*
LSD (0.05)	187.02	213.38	1.0	1.0	14.0	16.6	0.30	0.20

* Significantly different than the check at $P = 0.05$.

shorter fiber. None of the Acala cultivars carried BNL3590-190, while this SSR existed in 67% (12/16) of the non-Acala cultivars. For BNL3792-235 located on Chromosome A02 (Lacape et al., 2003), it was present in all the Acala cultivars, while it was absent in only four non-Acala cultivars. Three SSRs, BNL3590-183, BNL3590-190, and BNL3649-193, were significantly correlated with lower fiber strength. For the marker BNL3590-183, it was absent in most of the Acala cultivars (5/6), and present in 72% of the non-Acala cultivars (13/18). One SSR BNL3590-190 was significantly correlated with elevated micronaire readings. Of the six SSRs, only BNL3590-190 and BNL3649-193 were significantly correlated with more than two traits. Presence of BNL3590-190 was associated with reduced fiber length, strength, and higher micronaire readings, while BNL3649-193 was associated with increased lint yield and lower fiber strength.

Upon inspection of the origin of these SSRs by comparing upland cotton (including TM-1) and 3-79, the *G. barbadense* genetic standard, we found that all four SSR alleles of upland cotton origin were correlated with shorter, weaker, and coarser fiber. Two alleles (BNL3590-175 and BNL3590-185) from *G. barbadense* were correlated with better fiber quality, while the presence of two *G. barbadense* alleles (BNL1694-252 and BNL2634-254) in upland cotton was correlated with lower yield. However, presence of *G. barbadense* allele BNL3649-193 in upland cotton was correlated with yield improvement. The three New Mexico cultivars are typical Acala cottons with high fiber quality, and the California Acalas PHY 72 and PHY 78 also contained New Mexico Acala cotton germplasm in their pedigrees. These five germplasm resources as a group (Table 3) represent Acala-type cotton that contained significant *G. barbadense* germplasm introgression. Long-term selection and selfing for fiber quality in the Acala cotton breeding programs have retained the fiber quality genes and closely linked DNA markers that might be expected to show linkage disequilibrium. DP 491 and Australian-bred FM 989 have intermediate fiber quality and cotton yield when produced in New Mexico. The other four upland cotton cultivars did not indicate any *G. barbadense* germplasm introgression based on their pedigree information, representing non-Acala-type high-yielding (except for Atlas) germplasm. The differences between the two groups were profound in that the Acala cotton type had longer, stronger, and finer fiber (Table 5). Therefore, some of the *G. barbadense*-specific SSR marker alleles in the Acala germplasm could be associated with quantitative trait loci (QTL) for the fiber quality traits, while some of the other SSRs could be associated with QTL for cotton yield.

Although the present study was designed to assess the genetic diversity among commercial cultivars planted to 37% of U.S. cotton acreage in 2004, discrepancies in SSR frequencies between the two groups (Acala vs. non-Acala) were detected that were correlated with fiber yield and/or quality. Linkage disequilibrium association mapping has been successfully used in human genetics, and only recently applied in plants (Hansen et al., 2001; Flint-Garcia et al., 2003; Simko et al., 2004). Tenesa et al. (2003) proposed that selective genotyping (5%) could be powerfully used on a large number of unrelated germplasm lines for QTL mapping. The two groups in our study comprised of the high fiber quality and high yield groups could be considered as nonrandomly selected samples in the cotton germplasm pool.

Table 4. Correlation between fiber yield and quality traits based on field data from commercial cultivars tested near Las Cruces, NM, in 2001 and 2002.

Trait	Yield	Fiber length	Fiber strength	Micronaire
Yield	1			
Fiber length	−0.07	1		
Fiber strength	−0.39	0.77**	1	
Micronaire	0.23	−0.58	−0.64*	1

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Table 5. Correlation coefficient between presence of SSR markers and fiber yield and quality in commercial cultivars tested near Las Cruces, NM, in 2001 and 2002.

Marker	Chromosome or linkage group	Lint yield	Fiber length	Fiber strength	Micronaire
BNL1694-252	C7	−0.62*	−0.30	−0.04	0.09
BNL3590-183	unknown	0.54	−0.26	−0.76**	0.53
BNL3590-190	C2	0.36	−0.67*	−0.85**	0.70*
BNL3792-235	A02	0.04	−0.69*	−0.34	0.52
BNL2634-254	unknown	−0.68*	0.09	0.40	−0.22
BNL3649-193	D02	0.78**	−0.47	−0.63*	0.53

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Our results point to the necessity of exploring the extent to which linkage disequilibrium exists in cotton by using more markers covering the cotton genome and more germplasm lines randomly chosen from the cotton germplasm pool (Simko et al., 2004). Of course, as with most QTL discovery, validation of the marker–trait associations should be conducted using segregating populations and near-isogenic lines. These SSRs would have to be introgressed into various genetic backgrounds followed by comparison of near-isogenic lines among environments to derive a data set with sufficient rigor to confirm QTL effects and their interactions with backgrounds and environments. However, it should be pointed out that most of the QTL reported in crops, including cotton, have not yet been confirmed.

Quantitative trait loci mapping for cotton yield, yield components, and fiber quality traits has been reported in recent years (Kohel et al., 2001; Saranga et al., 2001; Guo et al., 2003; Mei et al., 2004; Paterson et al., 2003; Zhang et al., 2003). In these studies, since most of the segregating populations used were developed from interspecific crosses between upland cotton and *G. barbadense*, it should allow for identification of common chromosomal regions that might harbor QTL of agronomic importance if common DNA markers were utilized. However, the various laboratories used different DNA markers and parental lines for the development of mapping populations that each had their own set of polymorphic markers. Limited information on chromosomal locations and a limited number of DNA markers did not permit genome-wide mapping of the markers and QTL. As a result, the present study could not identify any fiber quality QTLs that were derived from *G. barbadense* by comparing the traits-associated SSR markers identified in the study with the previously reported results. Nevertheless, the putative fiber traits-associated SSR markers identified in the present study provides useful information for further investigations.

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